Method for detecting and quantifying first biopolymers that are located in a liquid

The invention relates to a method for detecting and quantifying first biopolymers that are located in a liquid.

It is known from the prior art that polynucleotide sequences, such as DNA, can be detected by voltammetric methods. To this end, it is proposed in US 5,312,572 to add redox-active molecules to the solution. In the case of hybridization, these molecules bind to the double-stranded molecule formed from the polynucleotide sequences. The redox-active molecule causes a measurable redox signal. A similar method is also disclosed in US 5,871,918.

WO 96/01836 discloses a chip for detecting polynucleotide sequences. A multiplicity of miniaturized reaction cavities are provided on the chip. A particular polynucleotide sequence is bound in each of the reaction cavities. On immersion of the chip into a solution containing the polynucleotide sequence to be detected, hybridization occurs with one of the particular polynucleotide sequences. The hybridization is detected by means of fluorescence.

WO 95/12808 also relates to a detection method using a chip. In this method, a voltage is applied across the chip in the manner of an electrode. Charged polynucleotide sequences that are located in the solution can thus be increased in concentration at the surface of the chip or at the miniaturized reaction vessels provided therein.

The prior-art methods are complex. They require the addition of particular redox-active molecules or the presence of chips which are complex to manufacture.

Precise quantification of the biopolymers to be detected is not possible using the known methods. Optical detection is usually necessary. This increases the apparatus complexity.

The object of the invention is to overcome the disadvantages of the prior art. In particular, the object is to indicate a method for detecting and quantifying biopolymers that are located in a liquid which can be carried out in the simplest and most inexpensive manner possible.

This object is achieved by the features of claim 1. Advantageous embodiments arise from the features of claims 2-14.

The invention proposes a method for detecting and quantifying first biopolymers that are located in a liquid in which second biopolymers which have a specific affinity to the first biopolymers to be detected are bound to the surface of a first electrode, and in which the first and at least one second electrode are in contact with the liquid, having the following steps:

a) application of a voltage and/or a current across the first and second electrodes, and

b) measurement of a direct change in the voltage and/or the current which is caused by the addition of the first biomolecules onto the second biomolecules.

The method proposed can be carried out simply and quickly. The change in the voltage and/or current is measured directly, i.e. it is not necessary to provide particular redox-active molecules. In particular, quantification of the first biopolymers that are

present in the liquid and are to be detected is also possible in a simple manner.

The applied voltage or current may be changeable, i.e. it may be alternating current. In step b, a direct-current signal can be measured, the measurement advantageously being carried out as a cyclovoltammetric measurement. In this case, the redox processes of the first biopolymers added onto the second biopolymers and Faraday electron transfer through the surface of the first electrode at a prespecified voltage or in a pre-specified voltage range are utilized.

For the detection and quantification of the first biopolymer, the measured current or the measured voltage is advantageously plotted against time and integrated at least over one peak. The integration is advantageously carried out with subtraction of the background. The amount of charge transferred through the adding-on of the first biopolymers can be determined from the value arising in the integration. This can be used to conclude the number of first biopolymers added on. Calibration is possible. The concentration of the first biopolymers to be detected in the liquid can be determined. The proposed method is particularly simple since here – as in conventional cyclic voltammetry – a linear voltage ramp is passed through cyclically only between the first electrode and a reference electrode. It is not necessary to employ redox-active molecules for charge transport.

The current flow can be measured via a third electrode or a counterelectrode. When the measured current or measured voltage is plotted against time, characteristic peaks can be observed which can be assigned to addition or adsorption and to desorption of the first biopolymers.

A further embodiment comprises measuring an alternating-current signal phase-sensitively. The addition of the first biopolymers onto the second biopolymers which are bound terminally to the first electrode causes a change in the double layer capacitance caused by the addition and repulsion of the charged biopolymers. The phase-sensitive measurement proposed enables the capacitive proportion of the alternating-current signal to be determined. Knowledge of the capacitive proportion allows conclusions to be drawn on the concentration of the first biopolymer to be detected in the liquid.

In this connection, it is possible to superimpose the alternating-current signal on a cyclic direct-current signal. According to a further embodiment of the method, the impendance is measured by measuring the voltammetric signals at varying frequency. This enables direct quantification of the degree of coverage of the surface of the first electrode by first biopolymers to be detected.

It is advantageous, before step a, to increase the concentration of the first biopolymers at the surface of the first electrode by applying a voltage and/or current. The increasing concentration is particularly effective if the polarity is reversed cyclically. Molecules which are not complementary to the second biopolymers are thereby repelled by the surface.

According to a further design feature, it is proposed that the first electrode with the first biomolecules increased in concentration at it is removed from the liquid and, optionally after carrying out a washing step, brought into contact with a defined measurement solution for the measurement. This enables interfering influences due to any further electrochemically active species that may be present in the liquid to be

substantially suppressed. Pre-measurement purification takes place to a certain extent.

The second biopolymer is advantageously bonded by means of one end to the surface of the first electrode via a covalent bond or via a linker. It is of course also possible for spacer molecules to be inserted in between for the bonding. The first electrode is advantageously made of one of the following materials: plastic, ceramic, glass or metal. In particular, polycarbonates and gold have proven particularly advantageous as electrode materials.

The terms first biopolymer and second biopolymer here are taken to mean, in particular, proteins, peptides, DNA, RNA and the like. The first biopolymer can be, in particular, a single-stranded DNA or RNA which is complementary to the second biopolymer. In step b, the change in voltage and/or current caused by the hybridization of the above-mentioned biopolymers is thus preferably measured.

The method is explained in greater detail with reference to working examples, in which

Fig. 1 shows a cyclic voltammogram, and

Fig. 2 shows the change in alternating current plotted against the frequency, and

Fig. 3 shows a diagrammatic view of a measurement set-up.

Example 1: Direct-voltage voltammetric measurement

Part of a DNA sequence (HGH1) which encodes for human growth hormone is bonded at its 5′ end to a first electrode E1 or working electrode made from poly-

carbonate/carbon fibers. The first electrode E1 dips into a solution containing 5 fM/µl of a complementary DNA sequence (HGH1 comp.), 80 mM TBE buffer and 100 mM NaCl conductive salt.

A voltage is increased in a linear manner from 0 to 1.3 V at a rate of change of 10 mV/s, then moved back to -1.3 V and finally up to 0 V again. The cyclic voltammogram measured during this operation is shown in Fig. 1. Two peaks are evident. In the anodic potential region, a first peak P1 occurs at U = 749.5 mV against Ag/AgCl, and in the cathodic potential region, a second peak P2 arises at U = -864.3 mV against Ag/AgCl. These peaks only occur if second biopolymers which are complementary to first biopolymers bound to the surface of the electrode are present in the solution. The peaks P1 and P2 are exclusively attributable to redox processes caused, in the present example, by hybridization of HGH1 with HGH1 comp. The first peak P1 located in the anodic region is the consequence of a redox process caused by adsorption or addition of HGH1 onto HGH1 comp. The second peak P2 which can be observed in the cathodic region can be associated with desorption of the hybridized nucleic acids. Integration of the first peak P1 over time corresponds to the amount of charge transported by the electrode surface. This enables conclusions to be drawn on the degree of hybridization on the surface of the first electrode.

Example 2: Alternating-current voltammetric measurement

The same electrode and solution are used as in Example 1.

A direct-voltage scan from 0 V to 1 V against Ag/AgCl is set up, and an alternating voltage of 250 Hz is superimposed on this direct voltage. The alternating current is measured in a phase shift of 90° to the

alternating voltage. If second biopolymers which are complementary to the first biopolymers that are located in the solution and to be detected are present on the first electrode, the peak shown in Fig. 2 arises at 250 mV. This peak is caused by a change in capacitance due to transport of negative charges to the surface of the first electrode during hybridization. The change in the double-layer capacitance results in a capacitive current flow, which can be detected by phase-sensitive measurement of the alternating-current fraction. The upper curve shows the alternating-current signal in the case of a first electrode with no biopolymer on the surface. No peak can be observed here.

Fig. 3 shows the measurement set-up in diagrammatic form. A first electrode E1, a second electrode E2 and a third electrode E3 dip into a liquid. The first electrode E1 is the working electrode. Second biopolymers, for example DNA, are covalently bonded thereto by means of a linker. First biopolymers (1) which are complementary to the second biopolymers (2) are located in the liquid. The second electrode (E2) serves as counterelectrode, the third electrode (E3) as reference electrode.

The following sequence listing shows the sequences of HGH1 and HGH1 comp.